

# Assessment of ROOT Dentin Translucency For age Estimation: The First Comparative Study of Conventional, Stereomicroscopic & Digital Methods.

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## ABSTRACT

**Background:** Introduction: Age estimation has always been a crucial concern in permissible and scandalous investigations for establishing one's identity. Assessment of chronological age of an individual by dental hard tissues is an important specialty in the turf of forensics especially in enigmatic conditions including mass disasters and festering postmortem residues. Teeth bestowing the properties of hardness, resilience prove to be the reliable material for age estimation in the identification of the unknown. Translucency in root dentin is considered to be one of the best criteria for estimation or assessment of dental age. **Objectives:** The present investigation evaluates and compares the effectiveness of conventional, stereomicroscopic and digital methods for age estimation by measuring root dentin translucency & concluding the best method among them. **Methods:** A total of 30 permanent teeth of the age group 21-80 years were sectioned longitudinally of thickness 250µm & translucency in root dentin was calculated using conventional, stereomicroscopic & digital methods and was compared. **Results:** There was no statistically considerable difference ( $p=0.584$ ) observed in translucency length obtained by the three methods. Linear regression equations derived from the three methods revealed most accurate method as digital followed by conventional and stereomicroscopic to assess age. The digital method is statistically significant with highest accuracy allowing better visualization, easy to use and less time consuming. **Conclusion:** Taking into consideration the benefits, the present study recommends the use of digital method to assess translucency for age estimation.

**Keywords:** Age estimation, identification, forensics, Gustafson, root dentin translucency, vernier caliper, stereomicroscope, scanner, adobe Photoshop.

## INTRODUCTION

Establishing individuality by estimating age using dentition has always been a primary concern for the forensic experts in forensic odontology in legal and criminal investigations. Assessment of chronological age of an individual by dentition for maintaining official and ethical records is of supreme significance. For identification purposes, biological profile of the person is to be renowned.<sup>[1]</sup> In unfathomable situations like decomposed

postmortem remains and mass disasters, the family & friends of the deceased cannot attest their identity as the human remains are not discernible. In such cases teeth lavishing the properties of hardness & resilience and its affinity to be least affected by the environmental factors,<sup>[2,3]</sup> nutrition & endocrinal turmoils gives teeth a unique property of permanency making it a reliable material for age estimation.<sup>[4]</sup>

Dental age is uniformly pertinent from infancy to tardy adolescence and is catalogued on the basis of developmental stages & degenerative changes along with the physiological development of the tooth.<sup>[5]</sup> In children, age can be estimated on the basis of tooth developmental stages but with advancing age, age assessment seems strenuous.<sup>[4]</sup> After procuring maturity, teeth endures various structural changes making age estimation possible in adults.<sup>[6]</sup> Gustafson's Morphohistologic approach considered six parameters for assessing age i.e.

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attrition, gingival recession, thickness of secondary dentin, cementum apposition, root resorption & Root dentin translucency. Among these root dentin translucency considered to be the best one for assessing age.<sup>[7]</sup> Caries, attrition & abrasion results in generation of stimuli causing blockage of dentinal tubules with hydroxyapatite crystals & collagen fibers resulting in translucent dentin. The translucency is seen because of the equalized refractive indices of the dentin in which tubules are occluded appearing translucent in transmitted light and dark in reflected light.<sup>[7-10]</sup> Translucency starts at the apical part and ascends towards the coronal part of the root with its symmetrical distribution in both side of the jaws.<sup>[8,11]</sup> The present investigation is designed to measure root dentin translucency for age estimation and correlating the measured translucency with the recognized age of individual. The present study also compares the effectiveness of conventional, stereomicroscopic and digital methods for estimating age by measuring root dentin translucency & concluding the best method among them.

## MATERIALS AND METHODS

This prospective study was conducted in the Department Of Oral Pathology, Microbiology & Forensic Odontology, Himachal institute of Dental Sciences, Paonta Sahib. 30 freshly extracted permanent teeth from thirty different individuals of age group 21-80 years were collected from the department of oral and maxillofacial surgery. Age and the cause of extraction were noted before extraction. Teeth extracted for periodontal problems, orthodontic purposes and prosthodontic purpose were included in the selection criteria followed by the exclusion of teeth with root caries, abrasion, erosion & root resorption. Teeth collected were kept in 10% Formalin in biopsy bottles & were given different sample numbers for the purpose of convenience during sectioning. After fixation for 24 hours, teeth were cleaned & the any soft tissue fragments, calculus, stains etc were removed. Buccolingual sections of the tooth were made of thickness 250 $\mu$ m by using micro motor and Arkansa stone. The sectioned samples were cleaned under running water and translucency in root dentin were measured by the three methods and compared.

- i. **Conventional Method:** Sectioned tooth samples of thickness 250  $\mu$ m were taken and first dried on the blotting paper. Sections were placed on view box. Beaks of digital vernier caliper were unmitigated from the apical limit to the coronal limit of translucency by placing the vernier caliper adjacent to the sectioned samples as shown in [Figure 1]. The measurements obtained were recorded

ii. **Stereomicroscopic Method:** The sampled sections were observed under stereomicroscope and a translucent graph paper was superimposed on the sections. The number of squares were counted depicting translucency from the apical zone to the translucent zone. One completely filled square and more than half completely filled square was taken as 1mm<sup>2</sup>, less than half filled squares were not counted. Translucency length was measured by counting the boxes and measurements were recorded as shown in [Figure 2].

iii. **Digital Method:** The sections were placed on a digital scanner and two scales were placed one horizontally (X-Axis) and other vertically (y-Axis) over a black sheet of paper with scanner's lid closed. The sections were scanned by keeping the lid closed for no passage of ambient light and for obstructing the light source to prevent appearance of dentin as a dark zone and rendering the section opaque over the black sheet of paper. The images were saved as JPEG files. The translucency was appeared as a light zone as the light source was obstructed. Images were imported to adobe photoshop computer based software for measuring the translucency length. The rulers were activated by clicking on rulers in the view option followed by placement of guides (horizontal) by clicking on the new guide option and filling the dimensions accordingly. To move a guide, move tool is used and the guide is moved to the desired location. Translucency length was measured by placing two horizontal guides starting from apical limit of translucency to the coronal limit of translucency. The distance between the guides is measured depicting the Maximum Translucency length (MTL) using the ruler tool on the tool box. Ruler tool is obtained by right clicking on the eyedropper tool. Select the ruler tool and a vertical line is drawn from between the guides giving the translucency length in mm. The results obtained were recorded and subjected to statistical analysis as shown in [Figure 3]

## Statistical Analysis

The measurements recorded for all the three methods were divided into 3 groups. The 1st group comprises of sectioned samples in which age estimation was done using conventional method, in 2nd group age was estimated using stereomicroscopic method followed by the 3rd group in which age estimation was done using digital method. ANOVA test was performed to evaluate the potential difference between the three methods and statistical analysis is done. Translucency measurements obtained by all the three methods were correlated to the known age using linear regression analysis. The Pearson's correlation coefficient with linear regression formula was derived for each group.

For conventional method/Group1, linear regression formula obtained was  
 $\text{Age} = -9.947 + (8.610 \times \text{translucency length})$

For stereomicroscopic method/Group 2, linear regression formula obtained was  
 $\text{Age} = -7.797 + (8.742 \times \text{translucency length})$

For digital method/Group3, linear regression formula obtained was  
 $\text{Age} = -8.9 + (8.430 \times \text{translucency length})$

## RESULTS

Results showed that in both methods the mean of the conventional method was 6.17 mm, for stereomicroscopic method mean came out to be 5.8 mm & for digital method was 6.18 mm. The ANOVA test also revealed no statistically significant differences between the three methods

( $P < 0.584$ ). Pearson's correlation and linear regression equations are shown in [Table 1]. Applications of linear regression equations for conventional method, stereomicroscopic and digital method are shown in [Tables 2,3,4]. The highest correlation was observed with digital method ( $r=0.942$ ), followed by conventional ( $r=0.936$ ) and stereomicroscopic methods ( $r=0.919$ ), where ( $r$ = correlation coefficient) as shown in [Table 5]. The linear regression formula derived revealed better ability of the digital method to assess age. Hence, the present study shows on comparing the known age (43.20) with estimated age by three different methods, the best method is Digital> Conventional> Stereomicroscopic method with the results 43.19 (highest accuracy)>43.18>42.9(least accuracy).

ANOVA test revealing no statistically significant difference ( $p=0.584$ ) in the translucency length obtained by the three methods.

Descriptives							
Translucency							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum
Conventional	30	6.1730	1.48877	.27181	5.6171      6.7289	3.03	8.73
Stereo	30	5.8333	1.44039	.26298	5.2955      6.3712	3.00	8.00
Digital	30	6.1800	1.53002	.27934	5.6087      6.7513	3.10	8.40
Total	90	6.0621	1.47902	.15590	5.7523      6.3719	3.00	8.73

Descriptive Statistics			
	Mean	Std. Deviation	N
Age	43.2000	13.69722	30
Stereo	5.8333	1.44039	30

### A. Conventional Method

**Table 2: Pearson's correlation coefficients (r) derived from conventional, digital & stereomicroscopic translucency Measurements**

	Mean	Std. Deviation	N
Conventional	6.1730	1.48877	30
Age	43.2000	13.69722	30
Correlations			
Conventional	Pearson Correlation		.936**
	Sig. (2-tailed)		.000
	N		30
Age	Pearson Correlation		.936**
	Sig. (2-tailed)		.000
	N		30

\*\*. Correlation is significant at the 0.01 level (2-tailed).

### B. Stereomicroscopic Method

Descriptive Statistics			
	Mean	Std. Deviation	N
Age	43.2000	13.69722	30
Stereo	5.8333	1.44039	30

Correlations			
		Age	Stereo
Age	Pearson Correlation	1	.919**
	Sig. (2-tailed)		.000
	N	30	30
Stereo	Pearson Correlation	.919**	1
	Sig. (2-tailed)	.000	
	N	30	30

\*\*. Correlation is significant at the 0.01 level (2-tailed).

### C. Digital Method

Descriptive Statistics				
	Mean	Std. Deviation	N	
Age	43.2000	13.69722	30	
Digital	6.1800	1.53002	30	
Correlations				
			Age	Digital
Age	Pearson Correlation	1	.942**	
	Sig. (2-tailed)			.000
	N		30	30
Digital	Pearson Correlation	.942**	1	
	Sig. (2-tailed)		.000	
	N		30	30

\*\*. Correlation is significant at the 0.01 level (2-tailed).

Table 3: Linear regression equations derived from conventional, digital & stereomicroscopic translucency Measurements

### A. Conventional Method

Coefficientsa						
Model	Unstandardized Coefficients			Standardized Coefficients Beta	t	Sig.
	B	Std. Error				
	(Constant)	-9.947	3.889		-2.558	.016
1	Conventional	8.610	.613	.936	14.045	.000

a. Dependent Variable: Age

Age: -9.947+(8.610 x translucency length)

### B. Stereomicroscopic Method

Coefficientsa						
Model	Unstandardized Coefficients			Standardized Coefficients Beta	t	Sig.
	B	Std. Error				
	(Constant)	-7.797	4.245		-1.837	.077
1	Stereo	8.742	.707	.919	12.364	.000

a. Dependent Variable: Age

Age: -7.797+(8.742 x translucency length)

### C. Digital Method

Coefficientsa						
Model	Unstandardized Coefficients			Standardized Coefficients Beta	t	Sig.
	B	Std. Error				
	(Constant)	-8.900	3.621		-2.458	.020
1	Digital	8.430	.569	.942	14.810	.000

a. Dependent Variable: Age

Age: -8.9+(8.430 x translucency length)

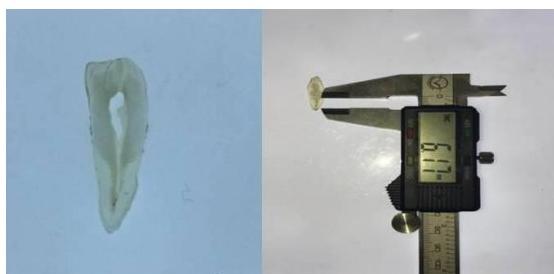


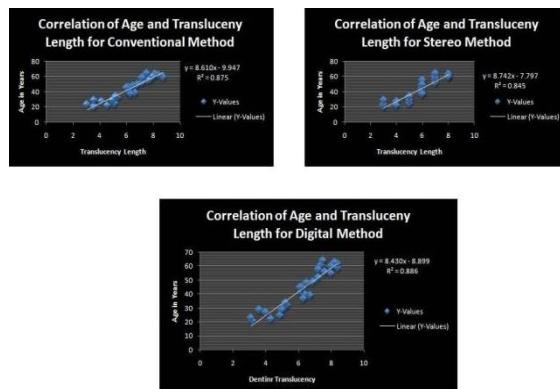
Figure 1: a. Image of the sectioned tooth sample imposed on the view box. b. The sectioned sample along with vernier caliper in which translucency length was measured by using the conventional caliper method



Figure 2: a. Ground section under a stereomicroscope. b. Ground section under a stereomicroscope over which a translucent graph paper was superimposed to count the no. of squares (1 mm<sup>2</sup>)



**Figure 3:** Scanned image of the tooth section using digital method Adobe Photoshop 7.0 version. The value obtained is the required translucency length (D1)



**Figure 4:** The diagrammatical relationship between translucency measurements between conventional, digital & stereomicroscopic methods & the correlation between the age and the translucency length.

## DISCUSSION

Age estimation in any medico-legal cases seems to be the primary step for identification of the unknown by forensic odontologist. Due to decrease in age estimation methods with increasing age by the use of bones, reliability of its assessment decreases. Need of the hour relies alternate methods for age estimation which are more infallible. For the identification of the deceased, utilization of teeth than bones because of its better preservation promotes it's to be the reliable parameter for age estimation. Gustafson reported six variables for age estimation out of which Root dentin translucency considered to be the best one.

With the advancement of age, occlusion of dentinal tubules results in mineralization which gradually increases the translucency of root dentin from root towards the crown.<sup>[4]</sup> Root dentin translucency starts from the apical limit of the root and extends towards the Cementoenamel junction.<sup>[1]</sup> Deceased periodontal tissue toxins, decreased dentinal tubular diameter and no functional stimuli proves to be major sources of translucency.<sup>[9,12,13]</sup> The visualization of ground sections reveals the translucency as a dark zone in reflected light and transparent in transmitted light. According to De Jonge, width of dentinal tubules decreases with advancing age from 3.2  $\mu$  (young individuals) to 1.2 $\mu$  (70 years).<sup>[1]</sup> Age estimation by root dentin translucency seems to be simple and easily applicable.<sup>[8]</sup>

Sectioned tooth samples for assessing length of root translucency are based on sliding caliper, stereomicroscopic and digital computer software methods.<sup>[14,11,15]</sup> In the present investigation ,sectioned teeth of 250 $\mu$ m were used and translucency length is measured using conventional, stereomicroscopic and digital methods and are compared. Also, the age derived was compared with the known age of an individual. In conventional caliper method, Root dentin translucency measured using digital vernier caliper on the teeth sectioned using disc bur and Arkansa stone. Translucency measurements are correlated to known age. Drawbacks in this method being no proper sterilization of caliper beaks, very less clarity between the translucent and opaque zone & increased risk of damage. In stereomicroscopic method, superimposition of translucent graph paper over the ground sections and counting the squares measuring 1 mm under a stereomicroscope were counted. Drawbacks' derived were increased intraobserver variability, less accuracy and increased chances of error. In digital method or a computer based method, Scanning of samples along with its editing in adobe Photoshop software using measure tool and guides & recording the translucency length obtained proves to be more efficient and accurate one. Translucency measurements obtained are magnified using the ZOOM TOOL allowing better visualization & concluding magnification is necessary for age estimation .The digital method is statistically significant with the highest accuracy for age estimation followed by the conventional and stereomicroscopic method. Intraobserver variability is very less and chances of error are less or nil. In case of Natural calamities and mass disasters, where identification of large number of bodies is required& large number of results are needed at one time. In such cases, Digital method rescues by scanning a large amount of sections at the same time and decreasing the time efficiency. Drusini et al.<sup>[16]</sup> studied age estimation by an image analysis system Integral Burst Alert System 2000 and caliper method revealing the predictive value of 45-48%. But this method proves to be expensive & time consuming. However an alternate method studied by Lopez Nicolas et al.<sup>[17]</sup> evaluated the image analysis systems for digital translucency. Computer based translucency measurements proves to be the best method for age estimation.

## CONCLUSION

In the present investigation, age estimation using linear regression equations showed better efficiency of the digital method to estimate age as compared to conventional & stereomicroscopic method. The highest correlation was observed with digital method( $r=0.942$ ), followed by

conventional( $r=0.936$ ) and stereomicroscopic methods( $r=0.919$ ). The present study is the first study where three methods of measuring root dentin translucency length were compared and the results were drawn. Also, on comparing the mean of the known age(43.20) with estimated age by three different methods, the best method was Digital>Conventional>Stereomicroscopic method with the results 43.19(highest accuracy)>43.18>42.9(least accuracy). The benefit of this study is that the digital method used is commercially available and widely used image editing software. So, the present study shows a significant correlation between the estimated age and known age. The linear regression formula derived gives an error of 2-3% less than the Gustafson's method (7-10% error) in estimating age. This can be due to small study group. If a larger study group along with different ethnic groups is compared, we may derive a formula better than the Gustafson's formula in future.

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